

#3

## Sorting by pools

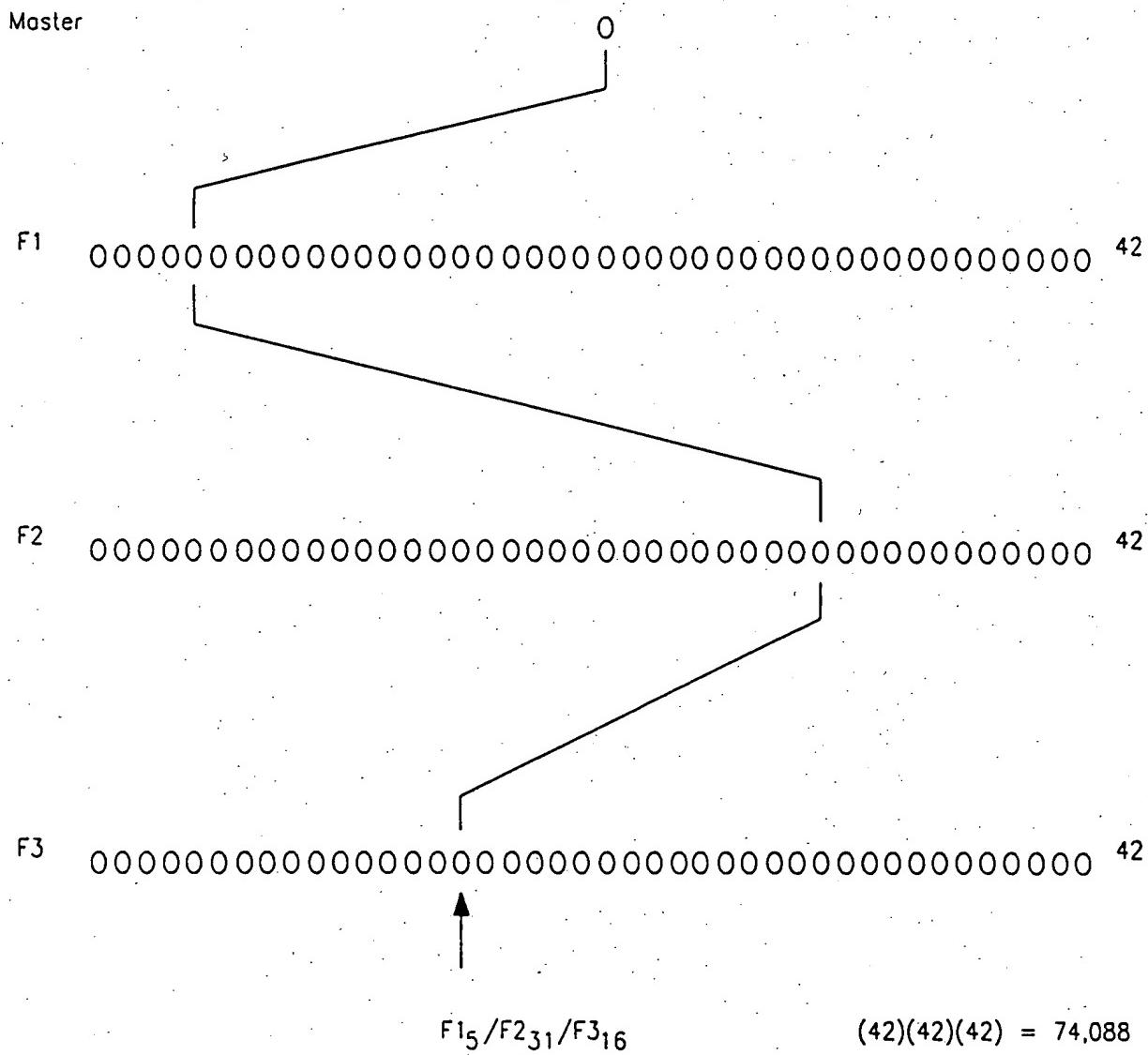


FIG. I

## Sorting by pools: Decreasing pool diversities

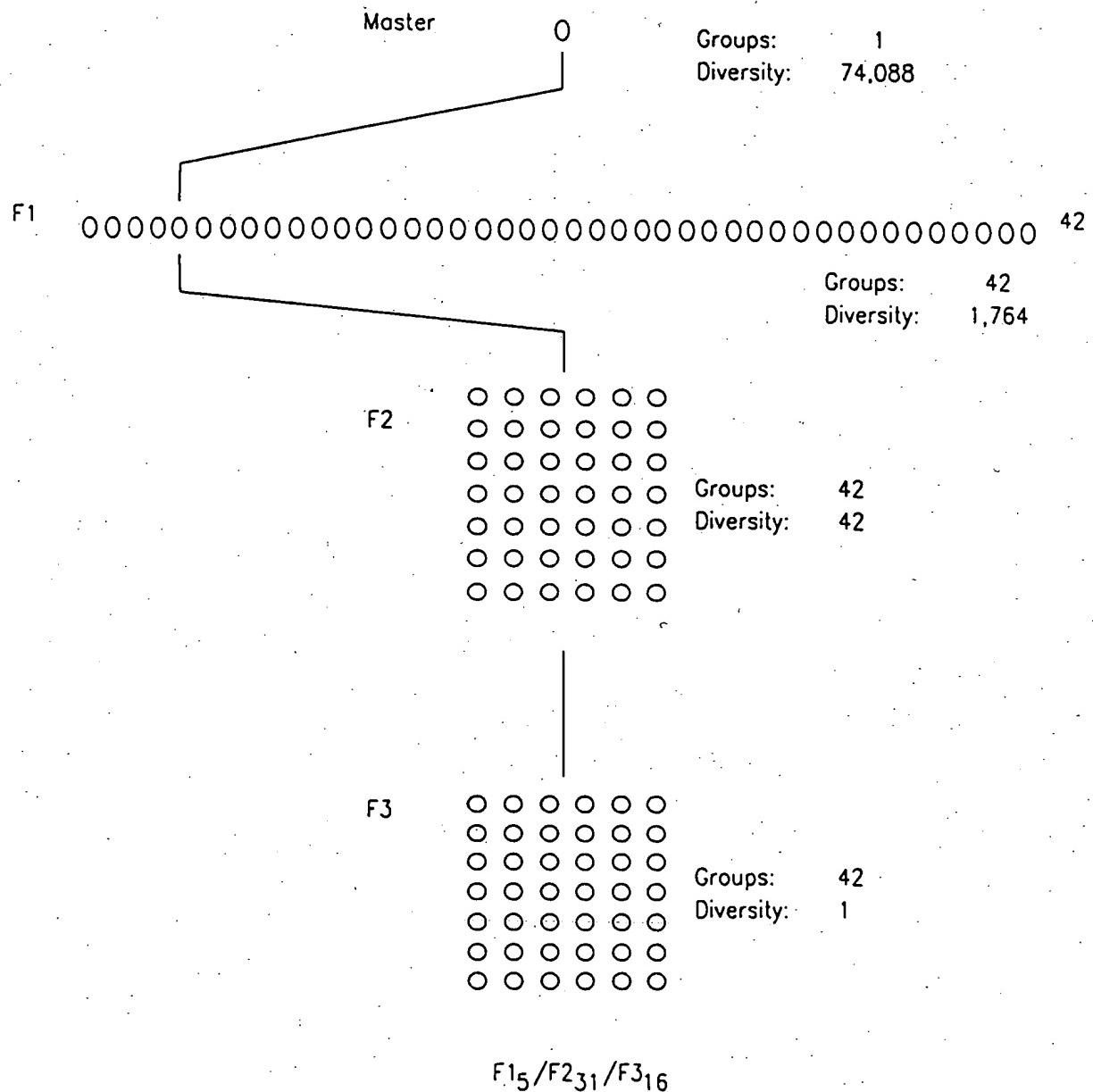


FIG. 2

Title: **COLLECTIONS OF BINDING PROTEINS AND TAGS  
AND USES THEREOF FOR NESTED SORTING AND  
HIGH THROUGHPUT SCREENING.**  
Applicant: Ault-Richter et al.  
Serial No. 09/910,120 Filed: July 18, 2001  
Our Docket No.: 25885-1751

## Sorting by pools: Screening large diversity libraries

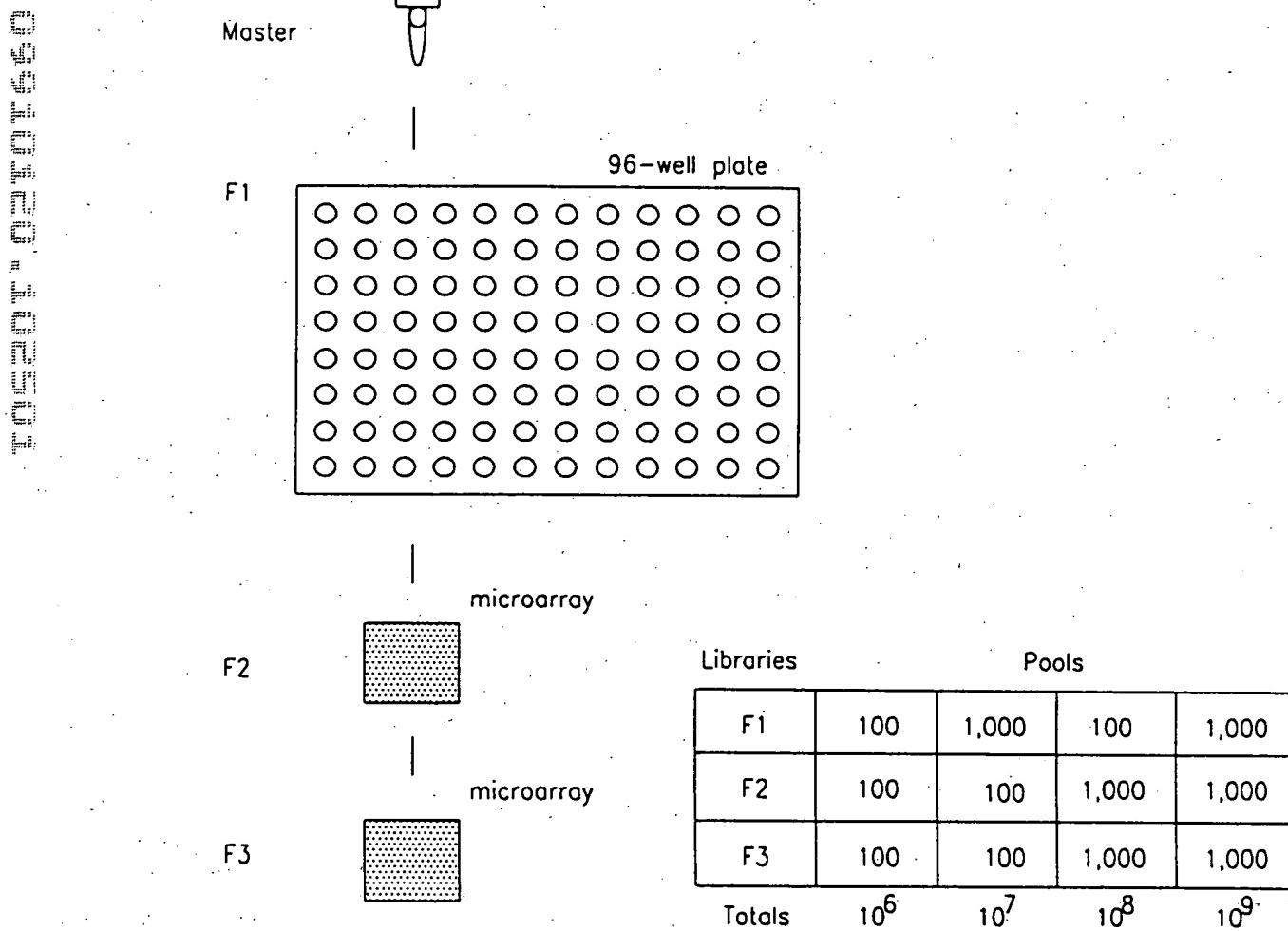


FIG. 3

## Searching a mutation library

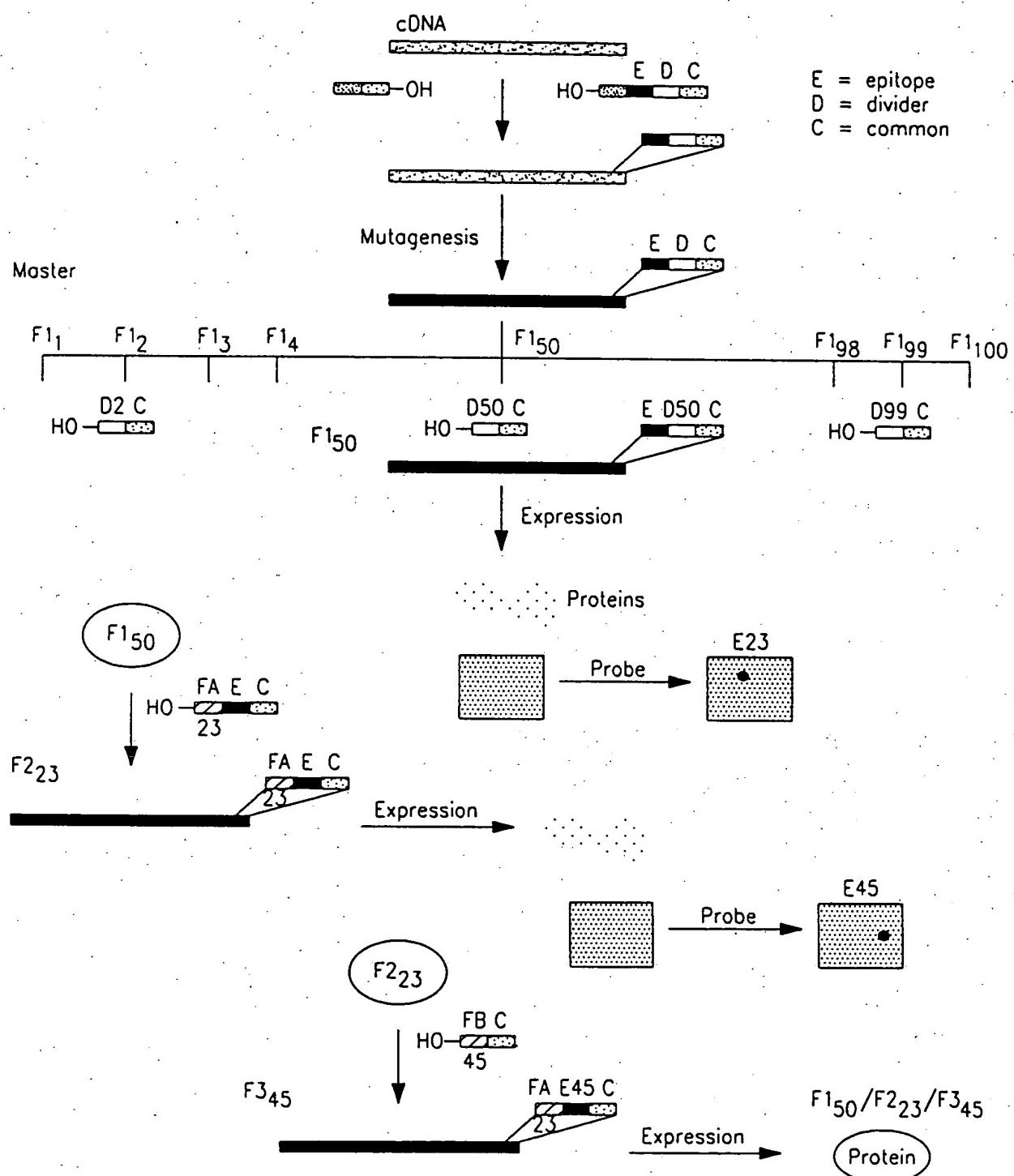
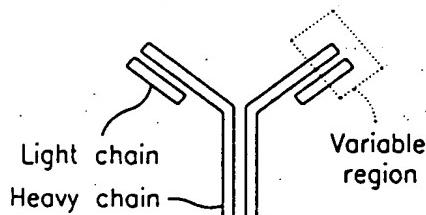


FIG. 4

Title: COLLECTIONS OF BINDING PROTEINS AND TAGS  
AND USES THEREOF FOR NESTED SORTING AND  
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Serial No. 09/910,120 Filed: July 18, 2001  
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## Making a recombinant antibody library



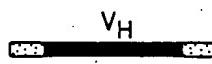
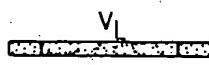
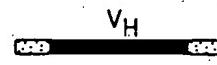
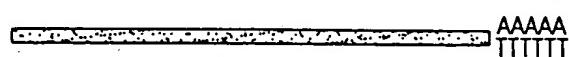
Spleen cells or PBLs



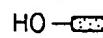
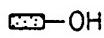
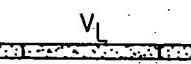
mRNA



cDNA



Linker



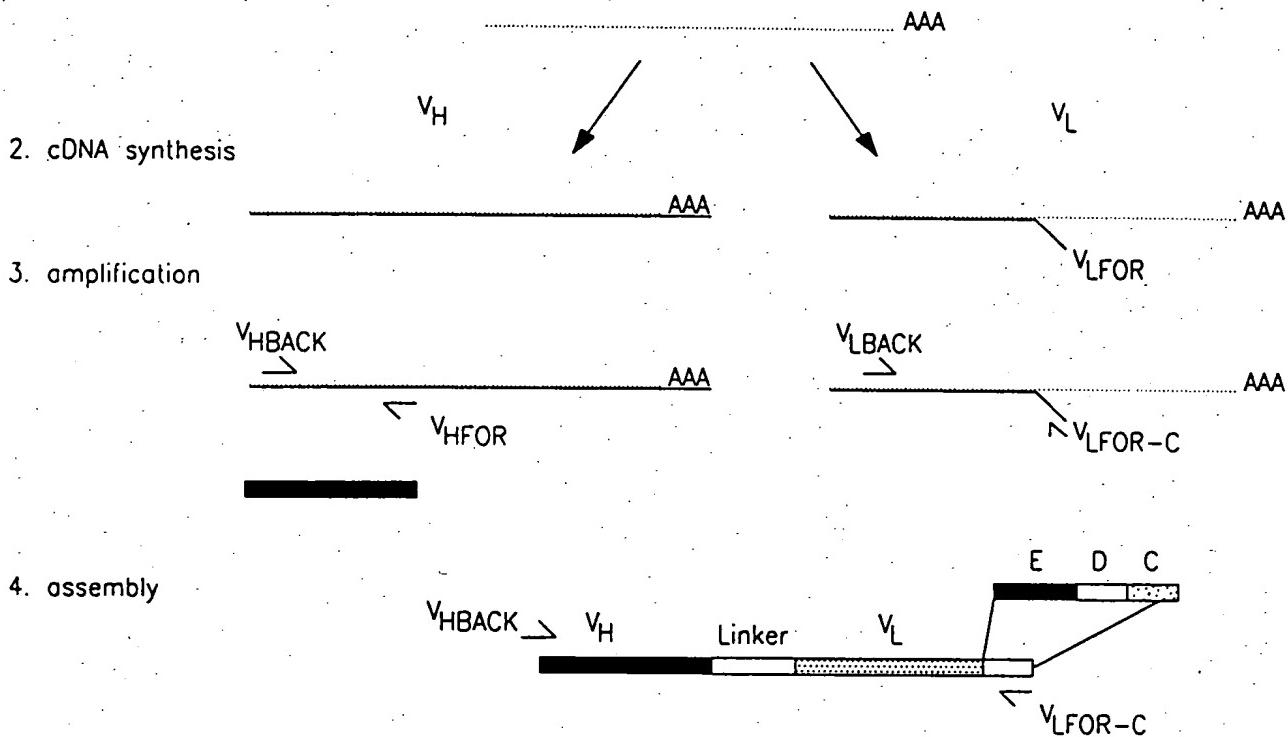
Expression

Antibodies

FIG. 5

## Creating the master antibody library: Primer incorporation

1. mRNA purification from spleen or PBLs



V <sub>H</sub> Primers		V <sub>L</sub> Primers	
Oligo dT	HO-TTTTTTTT(T) <sub>n</sub>	V <sub>LFOR</sub>	J <sub>kappa</sub> for E D C
	3' 5'		3' 5'
V <sub>HBACK</sub>	V <sub>H</sub> back OH	V <sub>LBACK</sub>	V <sub>kappa</sub> back OH
	5' 3'		5' 3'
V <sub>HFOR</sub>	OH J <sub>H</sub> for	V <sub>LFOR-C</sub>	C OH
	3' 5'		3' 5'

FIG. 6

## Creating the master antibody library: Linker addition

### 1. mRNA purification from spleen or PBLS

..... AAA

V<sub>H</sub>



### 2. cDNA synthesis

..... AAA

V<sub>L</sub>

AAA

V<sub>LFOR</sub>

### 3. amplification

V<sub>HBACK</sub>

AAA

V<sub>LBACK</sub>

AAA

V<sub>HFOR</sub>

Digest end and mix with V<sub>Linkers</sub>

V<sub>Linkers</sub>

Ligate and amplify

V<sub>LBACK</sub>

V<sub>LFOR-C</sub>

### 4. assembly

V<sub>HBACK</sub> V<sub>H</sub> Linker V<sub>L</sub>

E D C

V<sub>LFOR-C</sub>

#### V<sub>H</sub> Primers

Oligo dT HO-TTTTTTTT(T)<sub>n</sub>  
 3' 5'

V<sub>HBACK</sub> V<sub>H</sub> back OH  
 5' 3'

V<sub>HFOR</sub> J<sub>H</sub> for OH  
 3' 5'

#### V<sub>L</sub> Primers

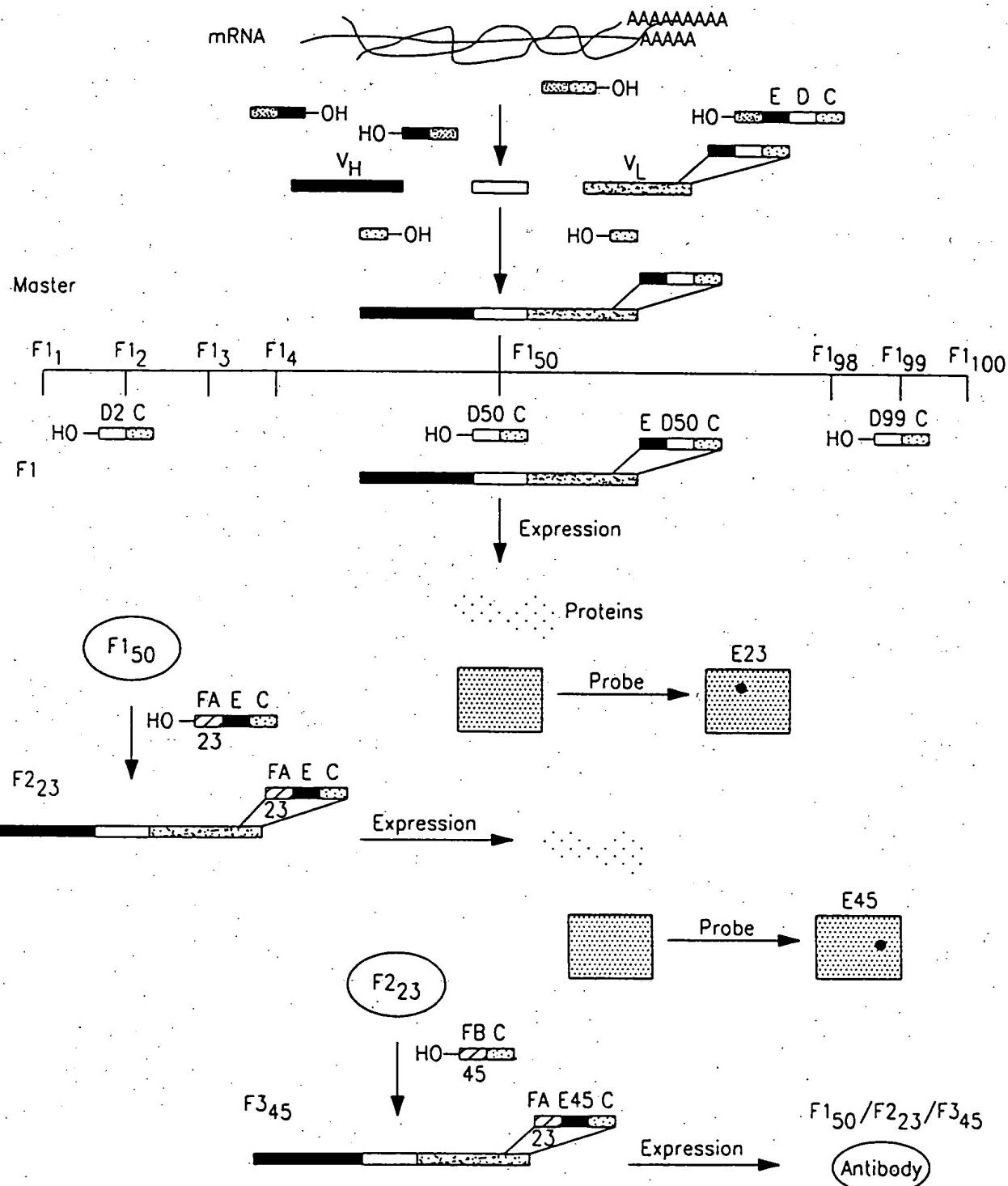
J<sub>kappa</sub> for HO ..... 5'  
 3'

V<sub>LBACK</sub> V<sub>kappa</sub> back OH  
 5' 3'

V<sub>Linkers</sub> J<sub>kappa</sub> for E D C  
 HO ..... 5'  
 C  
 3' 5'

FIG. 7

## Searching a recombinant antibody library



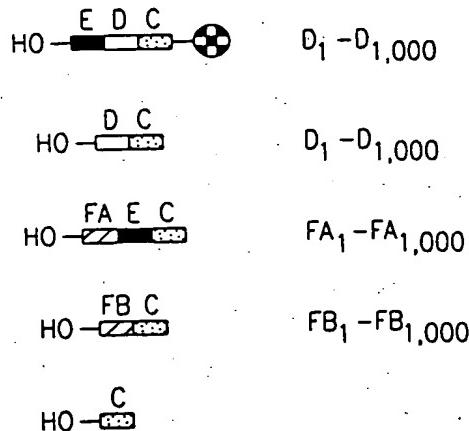
**FIG. 8**

Title: COLLECTIONS OF BINDING PROTEINS AND TAGS  
AND USES THEREOF FOR NESTED SORTING AND  
HIGH THROUGHPUT SCREENING.  
Applicant: Ault-Riche et al.  
Serial No. 09/910,120 Filed: July 18, 2001  
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## Physical elements to include in the kits and combinations

### • Anti-tag Arrays™

#### • Primer sets



#### • Readers

#### • Software

FIG. 9

Title: COLLECTIONS OF BINDING PROTEINS AND TAGS  
AND USES THEREOF FOR NESTED SORTING AND  
HIGH THROUGHPUT SCREENING.

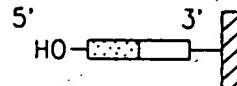
Applicant: Ault-Riche et al.  
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## Making the V<sub>LFOR</sub> primers: Solid phase synthesis

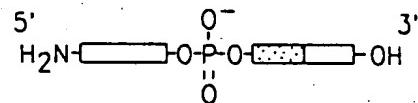


J kappa for      Epitope      D      Common

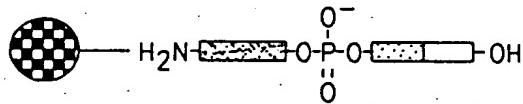
- Synthesize oligo on solid support



- Add aminolink prior to cleavage



- Couple to tosyl activated magnetic beads



- Extended by hybridizing with DNA patch and ligating

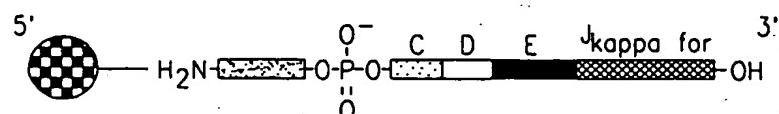
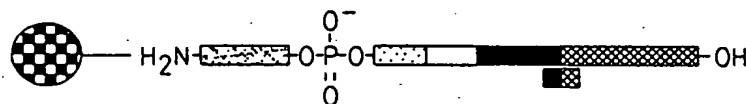
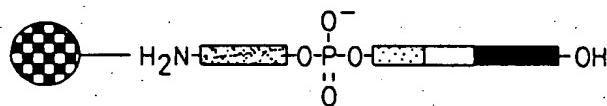
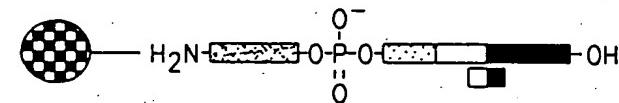
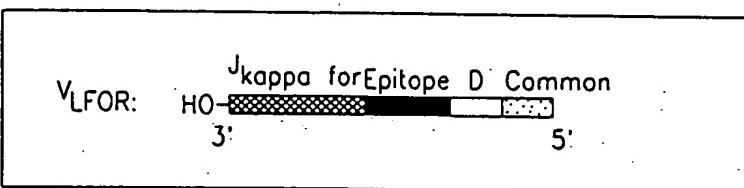


FIG. 10

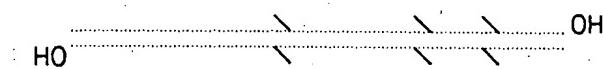
#### Making the V<sub>LFOR</sub> primers: Overlapping hybridization



$J_{\kappa}$  for Epitope D Common

- Synthesize 4,028 different oligos:  
(26 for  $\lambda$ <sub>kappa</sub> for : 2,000 for Epitope, 2,000 for D; 2 for Common

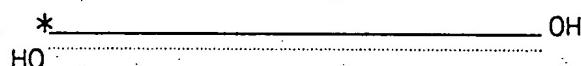
2. Assemble oligos for + and - strands of the different regions



- ### 3. Ligase the assembled oligos



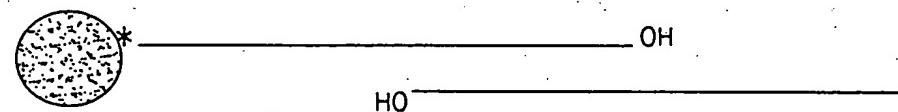
- #### 4. 1<sup>st</sup> strand synthesis with biotinylated primer



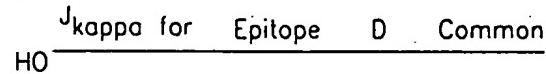
- 2<sup>nd</sup> strand synthesis with non-biotinylated primer.



6. Bind to avidin coated magnetic beads and then denature



- #### 7. Purify non-biotinylated ssDNA



## FIG. II

## Building the collection of antibody/tag pairs: Hybridoma screening

Stable hybridoma cells

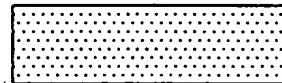
Isolated monoclonals  
grown in 96-well plates  
(quantify Abs in culture  
supernatants by ELISA)

Purified antibodies  
(purify with protein L plates;  
quantify purified antibodies by ELISA)

Individual protein  
preparations

Pooled protein  
preparations

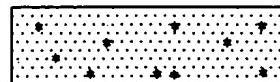
Array onto filter  
(up to 10,000 per filter)



Bind purified antibodies to  
magnetic beads coated with  
anti-mouse Ig mAb (Dynal)

Pan a random disulfide-constrained  
phage display library against the beads  
(4 rounds with plate amplifications)

Bind enriched phage library to filter;  
Stain with anti-phage mAb-HRP;  
Image with CCD-based system



Cut out best spots  
Recover and propagate phage  
(or PCR amplify DNA)

Sequence DNA to  
identify epitope tags

FIG. 12

Title: **COLLECTIONS OF BINDING PROTEINS AND TAGS  
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HIGH THROUGHPUT SCREENING.**

Applicant: Ault-Riche *et al.*  
Serial No. 09/910,120 Filed: July 18, 2001  
Our Docket No.: 25885-1751

**Table 3 Primers for PCR Amplification of Human Antibody Variable Regions (V genes)****1. V gene primary PCR****A. Human VH back primers (sense)**

HuVH1aBACK	5'-CAG GTG CAG CTG GTG CAG TCT GG-3'
HuVH2aBACK	5'-CAG GTC AAC TTA AGG GAG TCT GG-3'
HuVH3aBACK	5'-GAG GTG CAG CTG GTG GAG TCT GG-3'
HuVH4aBACK	5'-CAG GTG CAG CTG CAG GAG TCG GG-3'
HuVH5aBACK	5'-GAG GTG CAG CTG TTG CAG TCT GC-3'
HuVH6aBACK	5'-CAG GTA CAG CTG CAG TCA GG-3'

**B. Human JH forward primers (anti-sense)**

HuJH1-2FOR	5'-TGA CGA GAC GGT GAC CAG GGT GCC-3'
HuJH3FOR	5'-TGA AGA GAC GGT GAC CAT TGT CCC-3'
HuJH4-5FOR	5'-TGA GGA GAC GGT GAC CAG GGT TCC-3'
HuJH6FOR	5'-TGA GGA GAC GGT GAC CGT GGT CCC-3'

**C. Human V kappa back primers (sense)**

HuVk1aBACK	5'-GAC ATC CAG ATG ACC CAG TCT CC-3'
HuVk2aBACK	5'-GAT GTT GTG ATG ACT CAG TCT CC-3'
HuVk3aBACK	5'-GAA ATT GTG TTG ACG CAG TCT CC-3'
HuVk4aBACK	5'-GAC ATC GTG ATG ACC CAG TCT CC-3'
HuVk5aBACK	5'-GAA ACG ACA CTC ACG CAG TCT CC-3'
HuVk6aBACK	5'-GAA ATT GTG CTG ACT CAG TCT CC-3'

**C. Human V lambda back primers (sense)**

HuVλ1BACK	5'-CAG TCT GTG TTG ACG CAG CCG CC-3'
HuVλ2BACK	5'-CAG TCT GCC CTG ACT CAG CCT GC-3'
HuVλ3aBACK	5'-TCC TAT GTG CTG ACT CAG CCA CC-3'
HuVλ3bBACK	5'-TCT TCT GAG CTG ACT CAG GAC CC-3'
HuVλ4BACK	5'-CAC GTT ATA CTG ACT CAA CCG CC-3'
HuVλ5BACK	5'-CAG GCT GTG CTC ACT CAG CCG TC-3'
HuVλ6BACK	5'-AAT TTT ATG CTG ACT CAG CCC CA-3'

**D. Human J kappa forward primers (anti-sense)**

HuJκ1FOR	5'-ACG TTT GAT TTC CAC CTT GGT CCC-3'
HuJκ2FOR	5'-ACG TTT GAT CTC CAG CTT GGT CCC-3'
HuJκ3FOR	5'-ACG TTT GAT ATC CAC TTT GGT CCC-3'
HuJκ4FOR	5'-ACG TTT GAT CTC CAC CTT GGT CCC-3'
HuJκ5FOR	5'-ACG TTT AAT CTC CAG TCG TGT CCC-3'

**D. Human J. lambda forward primers (anti-sense)**

HuJλ1FOR	5'-ACC TAG GAC GGT GAC CTT GGT CCC-3'
HuJλ2-3FOR	5'-ACC TAG GAC GGT CAG CTT GGT CCC-3'
HuJλ4-5FOR	5'-ACC TAA AAC GGT GAG CTG GGT CCC-3'

**FIG. 13A**

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## 2. Linker fragment PCR

## F. Reverse JH for scFv linker (sense)

	—FR4 heavy —————	linker
RHuJH1-2	5'-CC ACC CTG GTC ACC GTC TCC TCA GGT GG-3'	
RHuJH3	5'-GG ACA ATG GTC ACC GTC TCT TCA GGT GG-3'	
RHuJH4-5	5'-GA ACC CTG GTC ACC GTC TCC TCA GGT GG-3'	
RHuJH6	5'-GG ACC ACG GTC ACC GTC TCC TCA GGT GG-3'	

## F. Reverse Vk for scFv linker (anti-sense)

	—FR1 light —————	linker
RHuV <sub>k</sub> 1aBACKFv	5'-GG AGA CTG GGT CAT CTG GAT GTC CGA TCC GCC-3'	
RHuV <sub>k</sub> 2aBACKFv	5'-GG AGA CTG AGT CAT CAC AAC ATC CGA TCC GCC-3'	
RHuV <sub>k</sub> 3aBACKFv	5'-GG AGA CTG CGT CAA CAC AAT TTC CGA TCC GCC-3'	
RHuV <sub>k</sub> 4aBACKFv	5'-GG AGA CTG GGT CAT CAC GAT GTC CGA TCC GCC-3'	
RHuV <sub>k</sub> 5aBACKFv	5'-GG AGA CTG CGT GAG TGT CGT TTC CGA TCC GCC-3'	
RHuV <sub>k</sub> 6aBACKFv	5'-GG AGA CTG AGT CAG CAC AAT TTC CGA TCC GCC-3'	

F. Reverse V<sub>λ</sub> for scFv linker (anti-sense)

	—FR1 light —————	linker
RHuV <sub>λ</sub> BACK1Fv	5'-GG CGG CTG CGT CAA CAC AGA CTG CGA TCC GCC ACC GCC AGA G-3'	
RHuV <sub>λ</sub> BACK2Fv	5'-GC AGG CTG AGT CAG AGC AGA CTG CGA TCC GCC ACC GCC AGA G-3'	
RHuV <sub>λ</sub> BACK3aFv	5'-GG TGG CTG AGT CAG CAC ATA GGA CGA TCC GCC ACC GCC AGA G-3'	
RHuV <sub>λ</sub> BACK3bFv	5'-GG GTC CTG AGT CAG CTC AGA AGA CGA TCC GCC ACC GCC AGA G-3'	
RHuV <sub>λ</sub> BACK4Fv	5'-GG CGG TTG AGT CAG TAT AAC GTG CGA TCC GCC ACC GCC AGA G-3'	
RHuV <sub>λ</sub> BACK5Fv	5'-GA CGG CTG AGT CAG CAC AGA CTG CGA TCC GCC ACC GCC AGA G-3'	
RHuV <sub>λ</sub> BACK6Fv	5'-TG GGG CTG AGT CAG CAT AAA ATT CGA TCC GCC ACC GCC AGA G-3'	

## 3. Pull-through primers for introduction of restriction sites\*

## G. Human VH back (Sfi)primers (sense)

	—FR1 heavy —————	linker
HuVH1aBACKSfi	5'-GTC CTC GCA ACT <u>GCG</u> <u>GCC</u> CAG <u>CGG</u> <u>GCC</u> ATG GCC CAG GTG CAG CTG GTG CAG TCT GG-3'	
HuVH2aBACKSfi	5'-GTC CTC GCA ACT <u>GCG</u> <u>GCC</u> CAG <u>CGG</u> <u>GCC</u> ATG GCC CAG GTC AAC TTA AGG GAG TCT GG-3'	
HuVH3aBACKSfi	5'-GTC CTC GCA ACT <u>GCG</u> <u>GCC</u> CAG <u>CGG</u> <u>GCC</u> ATG GCC CAG GTG CAG CTG GTG GAG TCT GG-3'	
HuVH4aBACKSfi	5'-GTC CTC GCA ACT <u>GCG</u> <u>GCC</u> CAG <u>CGG</u> <u>GCC</u> ATG GCC CAG GTG CAG CTG CAG GAG TCG GG-3'	
HuVH5aBACKSfi	5'-GTC CTC GCA ACT <u>GCG</u> <u>GCC</u> CAG <u>CGG</u> <u>GCC</u> ATG GCC CAG GTG CAG CTG TTG CAG TCT GC-3'	
HuVH6aBACKSfi	5'-GTC CTC GCA ACT <u>GCG</u> <u>GCC</u> CAG <u>CGG</u> <u>GCC</u> ATG GCC CAG GTA CAG CTG CAG CAG TCA GG-3'	

## H. Human J kappa forward (Not) primers (anti-sense)

	—FR4 light —————	
HuJ <sub>k</sub> 1FORNot	5'-GAG TCA TTC TCG ACT <u>TGC</u> <u>GGC</u> <u>CCC</u> ACG TTT GAT TTC CAC CTT GGT CCC-3'	
HuJ <sub>k</sub> 2FORNot	5'-GAG TCA TTC TCG ACT <u>TGC</u> <u>GGC</u> <u>CCC</u> ACG TTT GAT CTC CAC CTT GGT CCC-3'	

## H. Human J kappa forward (Not) primers (anti-sense)(continued)

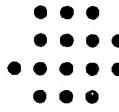
	—FR4 light —————	
HuJ <sub>k</sub> 3FORNot	5'-GAG TCA TTC TCG ACT <u>TGC</u> <u>GGC</u> <u>CCC</u> ACG TTT GAT ATC CAC TTT GGT CCC-3'	
HuJ <sub>k</sub> 4FORNot	5'-GAG TCA TTC TCG ACT <u>TGC</u> <u>GGC</u> <u>CCC</u> ACG TTT GAT CTC CAC CTT GGT CCC-3'	
HuJ <sub>k</sub> 5FORNot	5'-GAG TCA TTC TCG ACT <u>TGC</u> <u>GGC</u> <u>CCC</u> ACG TTT AAT CTC CAG TCG TGT CCC-3'	

## H. Human J lambda forward (Not) primers (anti-sense)

	—FR4 light —————	
HuJ <sub>λ</sub> 1FORNOT	5'-GAG TCA TTC TCG ACT <u>TGC</u> <u>GGC</u> <u>CCC</u> ACC TAG GAC GGT GAC CTT GGT CCC-3'	
HuJ <sub>λ</sub> 2-3FORNOT	5'-GAG TCA TTC TCG ACT <u>TGC</u> <u>GGC</u> <u>CCC</u> ACC TAG GAC GGT CAG CTT GGT CCC-3'	
HuJ <sub>λ</sub> 14-5FORNOT	5'-GAG TCA TTC TCG ACT <u>TGC</u> <u>GGC</u> <u>CCC</u> ACC TAA AAC GGT GAC CTG GGT CCC-3'	

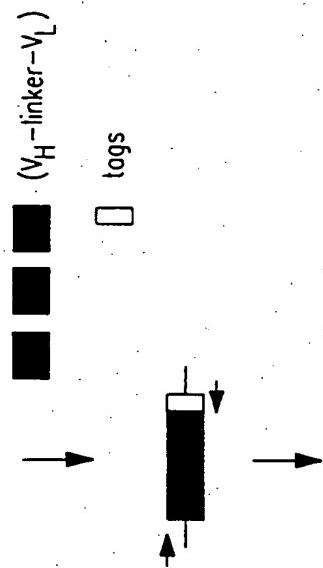
\*Recognition site for restriction enzyme is underlined.

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### step 1

Tag and assemble immunoglobulin genes



Create 1,000 sub-libraries by separate PCR amplification reactions using tag-specific PCR primers

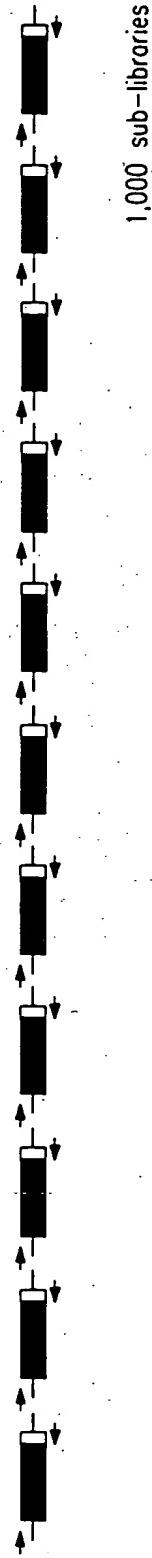


FIG. 14A

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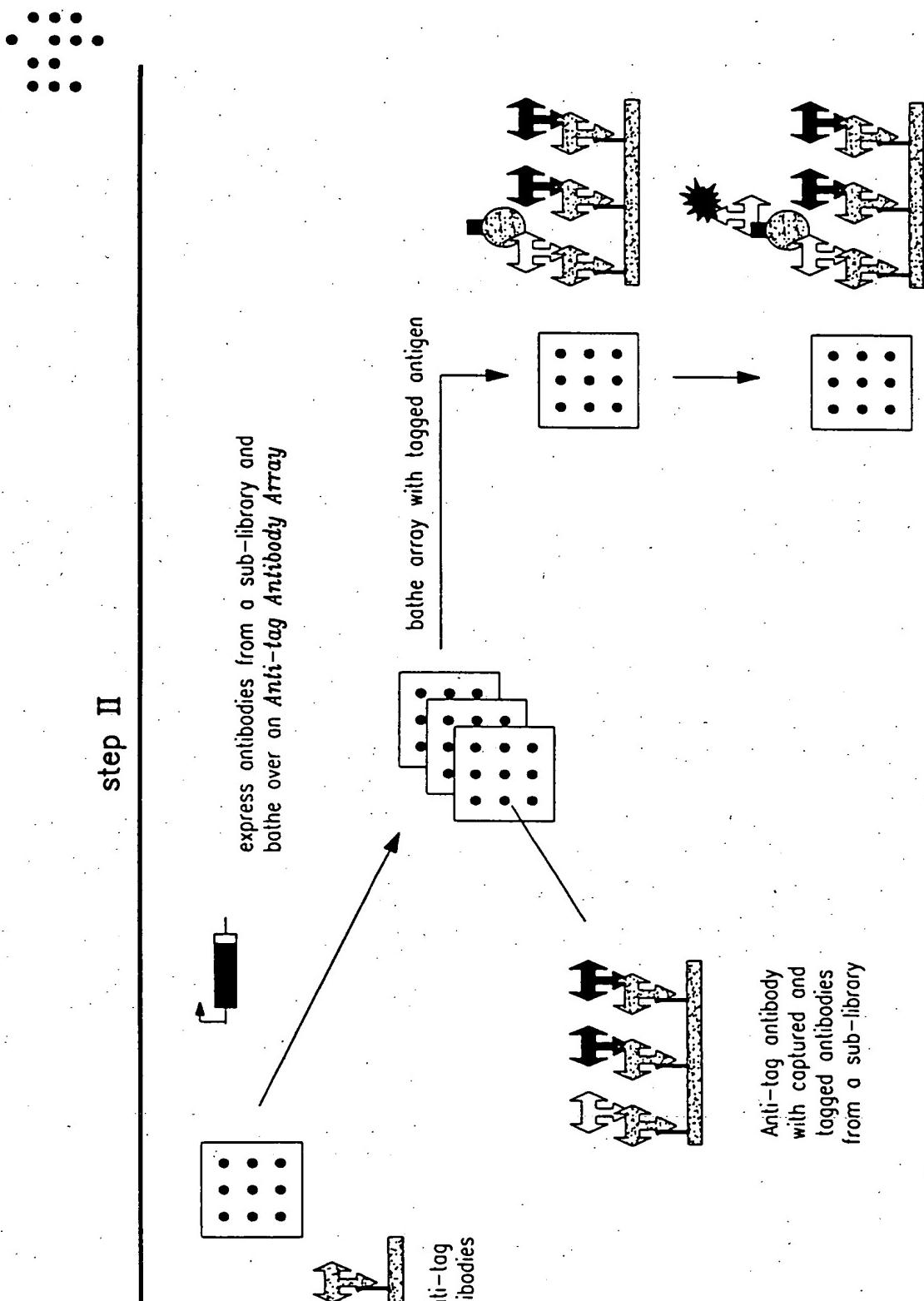
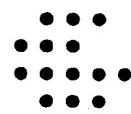


FIG. 14B

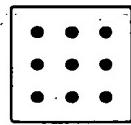
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### Step III

Amplify the antibody genes from the identified sub-library using tag-specific PCR primers



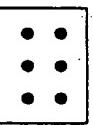
If the starting diversity of the master library was 1,000,000,000 then each spot in this array will have 1,000 different types of rAbs



Express and purify the antibodies



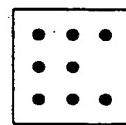
Re-distribute over an Anti-tag Antibody Array



If the starting diversity of the master library was 1,000,000,000 then each spot in this array will have a single type of rAb



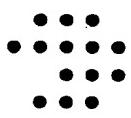
Re-survey to ID the antibody of interest



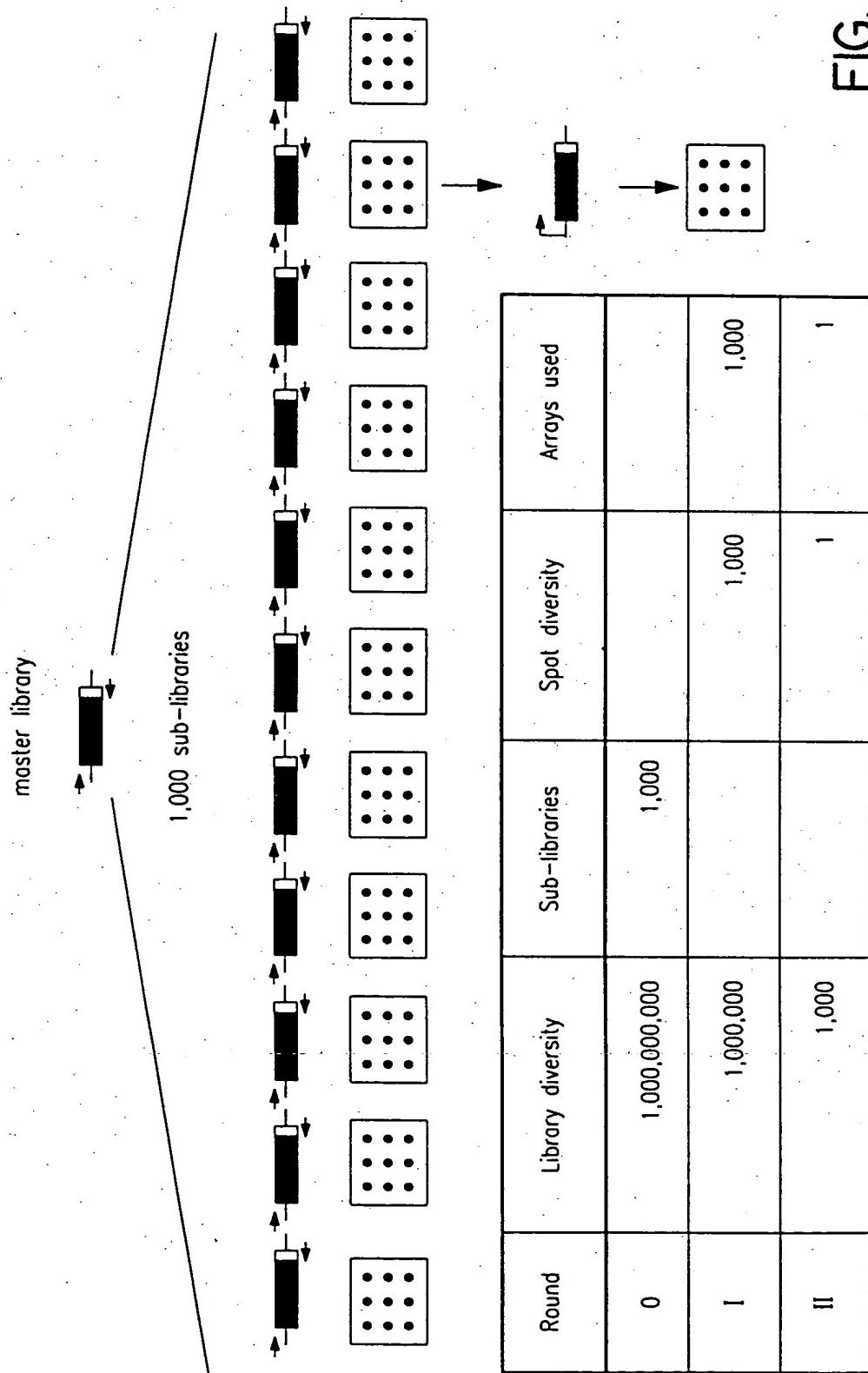
**FIG. 14C**

Title: **COLLECTIONS OF BINDING PROTEINS AND TAGS  
AND USES THEREOF FOR NESTED SORTING AND  
HIGH THROUGHPUT SCREENING.**

Applicant: Ault-Riche *et al.*  
Serial No. 09/910,120 Filed: July 18, 2001  
Our Docket No.: 25885-1751



## summary



**FIG. 14D**

Title: **COLLECTIONS OF BINDING PROTEINS AND TAGS  
AND USES THEREOF FOR NESTED SORTING AND  
HIGH THROUGHPUT SCREENING.**

Petitioner: Ault-Riche et al

Serial No. 09/910,120 Filed: July 18, 2001

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Modification searches

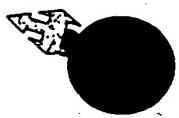
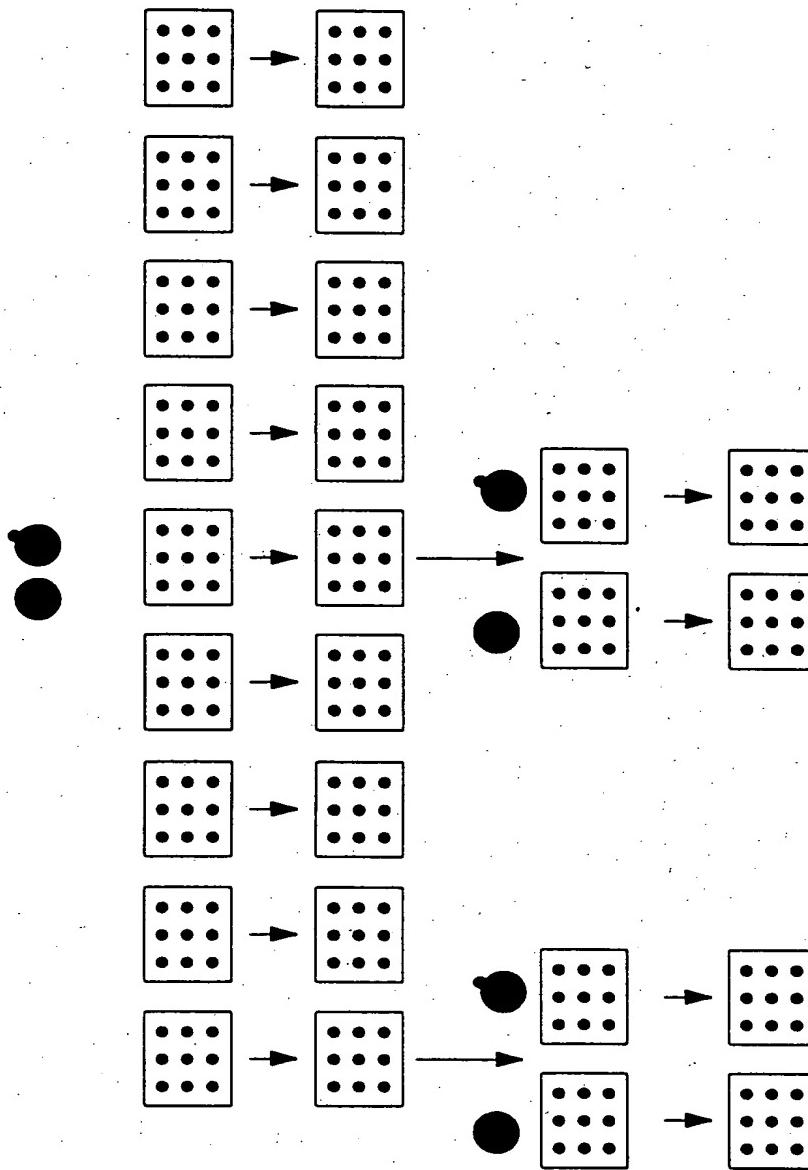
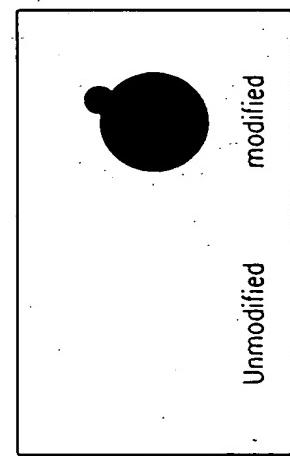
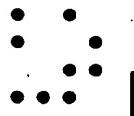
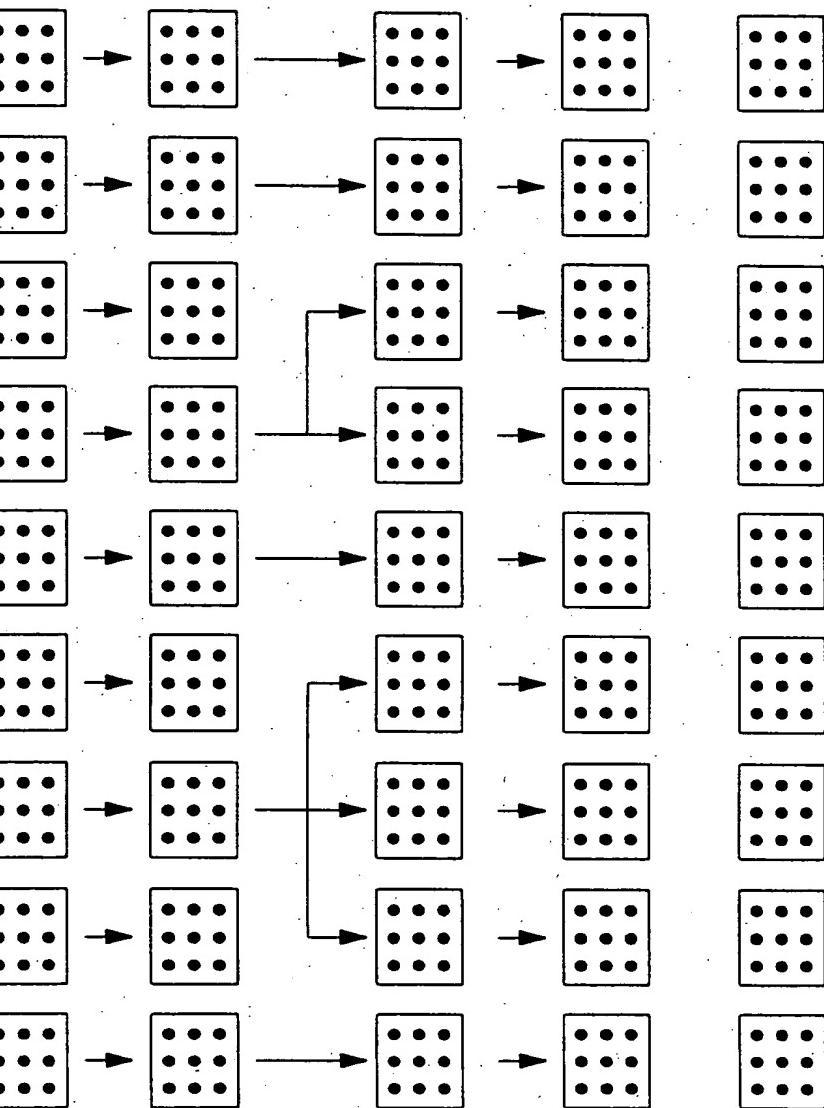


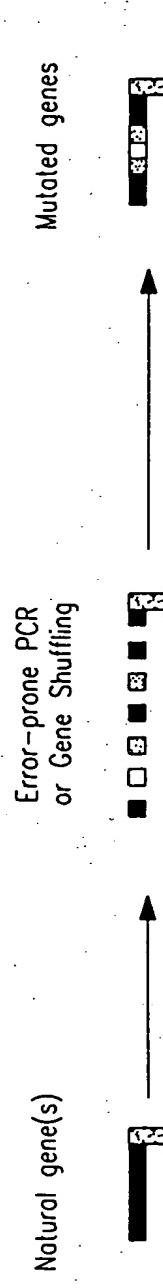
FIG. 5

Title: **COLLECTIONS OF BINDING PROTEINS AND TAGS  
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**FIG. 16****Simultaneous searches****Round Arrays Bait Probe****I 1,000 Abs Ags****II 1,000 Abs Ags****III  $\frac{1,000 \text{ Ags Abs}}{3,000}$** **3 Arrays per Ag**

## Protein interaction mapping

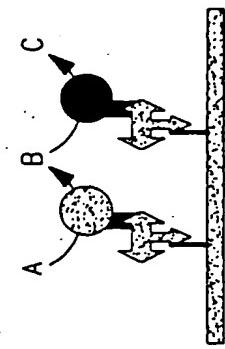


- tag the genes to be mutated

- mutate genes and create sub-libraries

- distribute mutants over arrays

- probe the arrays with labeled substrates



Spots can contain mixtures of enzymes  
for detection or pathway engineering

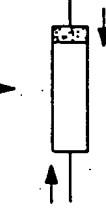
FIG. 17

## Protein interaction mapping

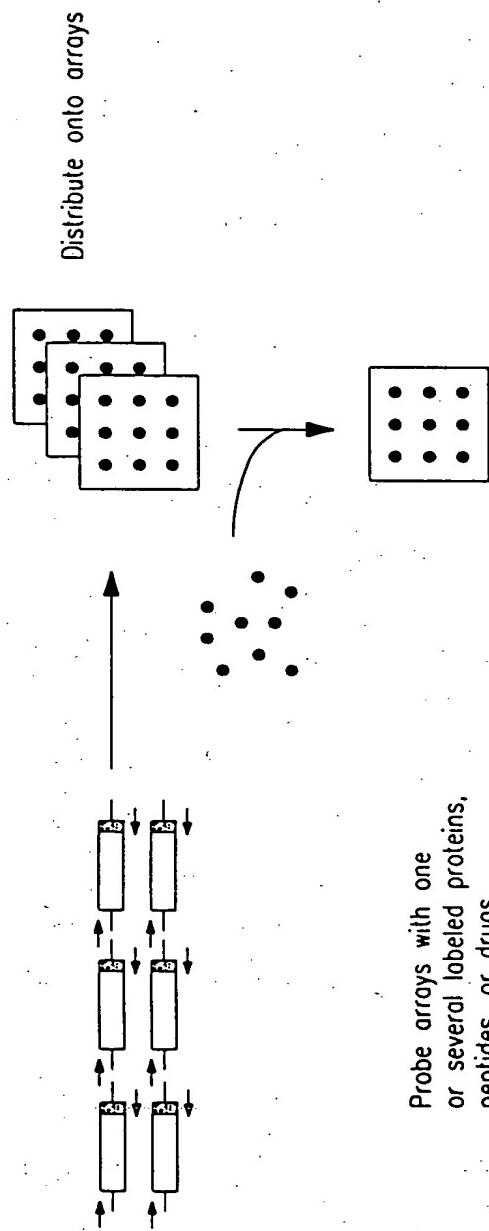
### cDNA library

- Human tissue
- pathogen
- yeast

AAAAAA  
Generate a tagged cDNA library



Create sub-libraries by PCR



Probe arrays with one  
or several labeled proteins,  
peptides, or drugs

FIG. 18

Title: **COLLECTIONS OF BINDING PROTEINS AND TAGS  
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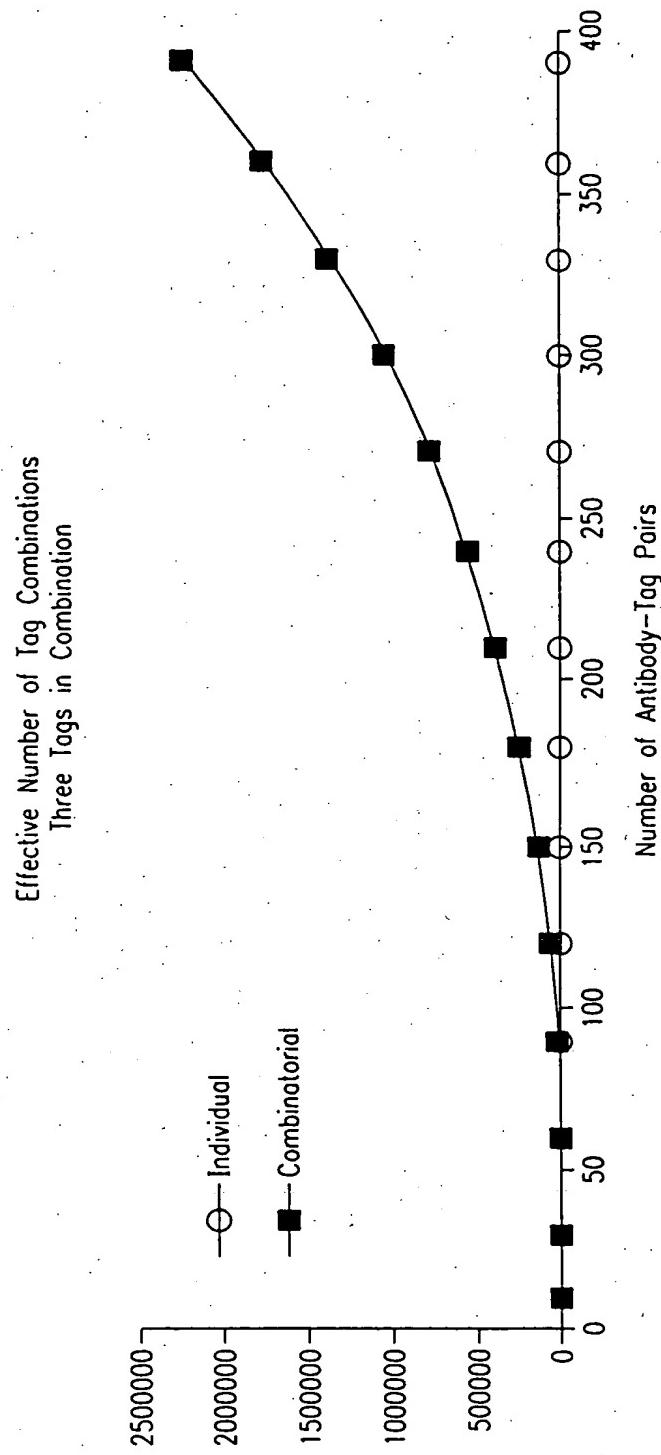


FIG. 19